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Paper Chromatography of 2,4-Dinitrophenylhydrazones

Resolution of 2-Alkanone, *n*-Alkanal, Alk-2-enal, and Alk-2,4-dienal Derivatives

A. M. GADDIS and REX ELLIS

Dairy and Meat Laboratory, Eastern Utilization Research and Development Division, Agricultural Research Service, U. S. Department of Agriculture, Beltsville, Md.

▶ A rapid paper chromatographic procedure for separating mixtures of 2,4-dinitrophenylhydrazones, 2-alkanones, *n*-alkanals, alk-2-enals, and alk-2,4-dienals of chain lengths up to 12 to 14 carbon atoms is described. Any member of the four homologous series studied, with the exception of acetone, ethanal, propanal, butanal, and propenal, can be identified. The procedure consisted of resolving into classes by ascending development with petroleum ether on untreated filter paper. Acetone separated with the *n*-alkanal class and methanal and ethanal with the alk-2-enal class. Earlier paper chromatography systems for the separation of *n*-alkanals were used to separate class groups into individual compounds. The inseparable mixtures were acetone-propanal, acetone-butanal, and ethanal-propenal.

A NUMBER of paper chromatographic systems are capable of separating a limited number of members of a homologous series of 2,4-dinitrophenylhydrazones. Ellis, Gaddis, and Currie (*3*) described a rapid ascending paper chromatographic procedure for separation of the C_1 to C_{14} *n*-aliphatic saturated aldehyde series, which was believed applicable to other homologous series of aliphatic monocarbonyls. However, it is extremely difficult to separate mixtures composed of different homologous series. Forss, Dunstone, and Stark (*5*) reported comparative R_f values of the four homologous series on the paper chromatographic system of Huelin (*9*) and Meigh (*15*); with the exception of acetone, values were similar for 2-alkanone C_n, alkanal C_{n+1}, alk-2-enal C_{n+2}, and alk-2,4-dienal C_{n+4}. Nonaka, Pippen, and Bailey (*17*) found a different R_f value alignment with the paper chromatographic system of Lynn, Steele, and Staple (*13*). Bassette, Day, and Keeney (*1*) separated homologous series easily on partition columns of nitromethane-hexane-Celite. However, mixtures of homologous series of 2-alkanones (except acetone), alkanals, and alk-2-enals were resolved into characteristic groups of C_n, C_{n+1}, and C_{n+2}, respectively. Monty (*16*) has reported a differential spectrophotometric determination of saturated aldehydes and ketones separated on a modified Kramer and van Duin (*12*) partition column. Pippen and coworkers (*18*, *19*) separated some of these inseparable groups with silicic acid–Celite adsorption columns, and by repeated chromatography of fractions isolated a number of aliphatic monocarbonyl derivatives from chicken tissue. Forss and Dunstone (*4*) have shown that adsorption columns are relatively destructive and may cause rearrangements.

This paper describes the extension of the paper chromatographic method of Ellis, Gaddis, and Currie (*3*) to other homologous series. A rapid new paper chromatographic method for the separation of mixtures of the four homologous series into classes is reported. The separation was referred to briefly by Gaddis and Ellis (*7*) and Ellis *et al.* (*8*) and this work was necessary to determine its effectiveness. The method (*8*) has since been used in a preliminary study of changes in the proportions of volatile monocarbonyl classes obtained from oxidizing fat.

SOLVENTS AND REAGENTS

ACS grades of carbon tetrachloride,

benzene, and methanol, and Skellysolve C (boiling point 91° to 95°) (Skelly Oil Co.) were purified as described (*3*). Technical grade petroleum ether was distilled, and the fraction boiling at 37° to 40° C. was used. Propylene glycol, U.S.P., and vaseline (white petroleum jelly, Blue seal, Chesebrough Manufacturing Co.) were also used.

MATERIALS AND EQUIPMENT

Materials and equipment have been described (*3*). Whatman No. 3 filter paper sheets were cut in the machine direction into tapering strips $10^1/_2 \times 1^3/_8 \times 1$ inches. Chromatographic chambers consisted of 300 × 38 mm. culture tubes. Mercury traps were used to regulate vapor pressure in the chromatographic chamber as described by Ellis, Gaddis, and Curry (*3*).

EXPERIMENTAL

Authentic C_1 to C_{14} alkanal 2,4-dinitrophenylhydrazones were prepared as described (*3*). Authentic C_5–C_{10} alk-2-enal 2,4-dinitrophenylhydrazones and C_5 to C_{12} alk-2,4-dienal 2,4-dinitrophenylhydrazones were used. C_3 and C_4 alk-2-enals were obtained from commercial sources. The C_{11} and C_{12} 2-enal homologs were prepared from C_9 and C_{10} saturated aldehydes by condensation with malonic acid (*6*), reduction with lithium aluminum hydride (*6*), and oxidation of the unsaturated alcohol by the method of Delaby and Guillot-Allègre (*2*). C_3 to C_9, C_{11} and C_{13} methyl ketone 2,4-dinitrophenylhydrazones were prepared from commercially obtained ketones. Hydrazone derivatives were prepared by the method of Iddles *et al.* (*10*), and purified by repeated recrystallization until a constant melting point was obtained.

Stock solutions in carbon tetrachloride of each hydrazone were prepared which contained the equivalent of approximately 25 mg. per liter (25 γ per ml.). Suitable volumes were taken from each stock solution to make up 100-ml. solutions of 30 μmoles per liter concentration. Aliquots of these solutions were used in the operations described below.

Paper chromatographic separations of homologous series were made as described (*3*). Various mixtures were prepared by mixing equal volumes of the proper solutions (30 μmole per liter concentration). The following formula was used to determine the volume of solution to spot a paper strip.

$$x \text{ ml.} = \frac{1.5\,n}{6\,ay}$$

where n = number of compounds in the mixture, a = absorbance at maximum of the solution, $y = 1$ for saturated aldehydes and methyl ketones, and $y = 2$ for alk-2-enals and alk-2,4-dienals. The capacity of the paper was much lower for 2-enals and 2,4-dienals, and a further reduction in the quantity applied was necessary, in addition to the decrease afforded by their higher extinction coefficients. Benzene was used in spotting the paper strips.

Resolution of mixtures of different homologous series was achieved by ascending solvent development on untreated Whatman No. 3 paper strips with 5.00 ml. of petroleum ether. The technique was otherwise the same, in every respect, as the method of separation of a homologous series (*3*). Because of the volatility of the solvent, chromatograms were usually run at 4° C. However, successful separations were obtained at temperatures as high as 20° C. Chromatograms were complete in about $1^1/_4$ hours. Amounts spotted on the paper were calculated by the simplified formula:

$$x \text{ ml.} = \frac{1.5}{a}$$

Separated spots were extracted for spectrophotometric measurements or application to other systems. The methods used and precautions necessary to remove impregnating agents have been described (*3*).

Table I. Paper Chromatography of 2-Alkanone, Alk-2-enal, and Alk-2,4-dienal Classes

2-Alkanone 2,4-dinitrophenylhydrazones	R_F[a] I Amount, γ	II Mixture	III Individual	IV Single extd. from I	V Mixture II extd. and authentic
Propylene glycol (20%) and Skellysolve-methanol (System 1)					
C_3	2.88	0.63	0.63	0.64	0.63
C_4	3.05	0.81	0.81	0.81	0.80
C_5	3.22	0.94	0.94	0.92	0.92
Alk-2-enal 2,4-dinitrophenylhydrazones					
C_3	0.99	0.38	0.38	0.39	0.39
C_4	1.05	0.49	0.50	0.48	0.50
C_5	1.11	0.67	0.67	0.67	0.68
C_6	1.17	0.80	0.80	0.82	0.83
C_7	1.23	0.92	0.92	0.92	0.93
Alk-2,4-dienal 2,4-dinitrophenylhydrazones					
C_5	0.73	0.45	0.46	0.45	0.46
C_6	0.77	0.56	0.55	0.56	0.56
C_7	0.81	0.73	0.73	0.74	0.74
C_8	0.85	0.85	0.85	0.85	0.85
C_9	0.89	0.94	0.95	0.93	0.95
Vaseline (7%) and aqueous methanol (System 2)					
2-Alkanone 2,4-dinitrophenylhydrazones					
C_6	3.39	0.72	0.72	0.73	0.72
C_7	3.56	0.65	0.65	0.65	0.65
C_8	3.72	0.59	0.60	0.60	0.58
C_9	3.89	0.51	0.51	0.52	0.51
C_{11}	4.23	0.36	0.38	0.37	0.36
C_{13}	4.60	0.24	0.24	0.24	0.24
Alk-2-enal 2,4-dinitrophenylhydrazones					
C_7	1.23	0.76	0.73	0.73	0.73
C_8	1.29	0.70	0.68	0.68	0.68
C_9	1.35	0.65	0.62	0.61	0.62
C_{10}	1.41	0.57	0.55	0.55	0.54
C_{11}	1.47	0.49	0.46	0.46	0.46
C_{12}	1.53	0.42	0.39	0.39	0.39
Alk-2,4-dienal 2,4-dinitrophenylhydrazones					
C_9	0.89	0.67	0.67	0.66	0.66
C_{10}	0.93	0.61	0.61	0.60	0.60
C_{11}	0.97	0.54	0.55	0.55	0.55
C_{12}	1.01	0.47	0.47	0.46	0.47

[a] Based on averages of 3 or more paper strips.

RESULTS AND DISCUSSION

Table I shows the R_F values obtained in the separation of homologous series of 2-alkanones, alk-2-enals, and alk-2,4-dienal 2,4-dinitrophenylhydrazones on propylene glycol–Skellysolve C–methanol and vaseline–aqueous methanol

Table II. Comparative R_F's of Four Homologous Series

	Paper Chromatography							
	Propylene Glycol				Vaseline			
R_F Values	2-Alkanone C_3–C_5	Alkanal C_1–C_6	Alk-2-enal C_3–C_7	Alk-2,4-dienal C_5–C_9	2-Alkanone C_6–C_9 C_{11}–C_{13}	Alkanal C_7–C_{14}	Alk-2-enal C_7–C_{12}	Alk-2,4-dienal C_9–C_{12}
1.00								
0.98								
0.96								
0.94				C_9				
0.92	C_5							
0.90		C_6	C_7					
0.88								
0.86								
0.84				C_8				
0.82								
0.80	C_4							
0.78		C_5	C_6					
0.76								
0.74				C_7		C_7	C_7	
0.72					C_6			
0.70							C_8	C_9
0.68			C_5			C_8		
0.66					C_7			
0.64		C_4					C_9	C_{10}
0.62	C_3					C_9		
0.60					C_8			
0.58								
0.56				C_6		C_{10}	C_{10}	C_{11}
0.54								
0.52			C_4		C_9			
0.50		C_3						
0.48							C_{11}	C_{12}
0.46						C_{11}		
0.44								
0.42				C_5			C_{12}	
0.40						C_{12}		
0.38					C_{11}			
0.36			C_3					
0.34		C_2						
0.32						C_{13}		
0.30								
0.28								
0.26						C_{14}		
0.24					C_{13}			
0.22		C_1						
0.20								
0.18								
0.16								
0.14								
0.12								
0.10								

systems. The separation was evaluated as described (*3*) for saturated aldehydes.

Table II compares the R_F values of individual compounds separated from homologous series of the four classes of monocarbonyl 2,4-dinitrophenylhydrazones. The closeness of R_F values of 2-alkanone C_n, alkanal C_{n+1}, alk-2-enal C_{n+2}, and alk-2,4-dienal C_{n+4} on the propylene glycol–Skellysolve C–methanol system agrees with the observations of Forss, Dunstone, and Stark (*5*) and follows the characteristic inseparable group pattern described by Bassette, Day, and Keeney (*1*). The R_F values indicated that mixtures should be separable into these characteristic groups. A mixture of 2-alkanones C_4 to C_5, alkanals C_2 to C_6, alk-2-enals C_3 to C_7, and alk-2,4-dienals C_5 to C_9, theoretically separable to five groups, separated into five spots on propylene glycol–impregnated paper. The spots were large and closely spaced and had R_F values of 0.93, 0.82, 0.68, 0.52, and 0.36. This separation was not further investigated.

The R_F alignment on the vaseline–aqueous methanol system was different. Those compounds with closely similar R_F values had the pattern of methyl ketone C_n, saturated aldehyde C_{n+2}, 2-enal C_{n+2}, and 2,4-dienal C_{n+3}. However, the spacing was so close on this system that a full mixture of all four classes would be very difficult to separate into characteristic groups.

The resolution of combinations of single compounds from each of the four classes is shown in Table III. Evaluation of the separation into classes was made by determination of the absorption maxima and rechromatography on propylene glycol or vaseline-impregnated paper with simultaneous determinations of the R_F values of individual authentic compounds. Results indicate the capabilities and limitations of the paper chromatographic method for separation into classes. The method did not separate saturated aldehydes C_1 or C_2 from 2-enal C_3, or any other 2-enal. Similarly, methyl ketone C_3 (acetone) was not separated from saturated aldehydes C_3 or C_4 or any higher molecular weight saturated aldehyde. Acetone, however, was readily separated from saturated aldehydes C_1 or C_2. In the absence of methyl ketone C_3 and saturated aldehydes C_1 and C_2, the method apparently will separate any combination of compounds of the four classes, including the characteristic inseparable groups (*1*).

Saturated aldehyde C_1 and 2-enals, inseparable by the class method, were readily separated on the propylene glycol–Skellysolve C–methanol paper chromatographic system. However, saturated aldehyde C_2 could not be separated from 2-enal C_3 on that system. Similarly, acetone was not separated from either propanal or butanal on the propylene glycol system.

Table IV shows the resolution of a mixture of four homologous series in the absence of acetone, methanal, and ethanal. Evaluation of the resolution into classes was made by separation of each class into individual compounds and identification with authentic mixtures. The value of the total procedure in the rapid identification of appropriate unknown mixtures is obvious.

Similar data are given in Table V for a complete mixture of four homologous series. The presence of acetone, methanal, and ethanal derivatives in a mixture of 2,4-dinitrophenylhydrazones can be detected by their tendency to partition between carbon tetrachloride and a 2*N* hydrochloric acid solution of 2,4 - dinitrophenylhydrazine (*8*). The behavior of acetone, methanal, and ethanal was as indicated in the preceding study of mixtures of single members of the homologous series (Table III). Acetone separates in the saturated aldehyde class, and methanal and ethanal in the 2-enal class. Complications from this resulted in the formation of an ethanal-propenal mixture and apparent acetone-propanal and acetone-butanal mixtures. The data in Tables III and V strongly suggest a distribution of acetone between propanal and butanal. Application of alcoholic alkali fading studies (*3*, *11*) and the method of Monty (*16*) should be useful in the detection and determination of such mixtures. The binary mixtures are theoretically separable by repeated chromatography and, among the many paper chromatographic methods (*3*, *14*) for separation of 2,4-dinitrophenylhydrazones, there are doubtless several capable of resolving these mixtures (*17*).

Table III. Resolution of Individual Members of Homologous Series

Mixture	Max., $M\mu$ (CCl_4) Authentic Compounds	Paper Chromatography Untreated Paper-Petm. Et_2O. Sepn. Mixture of Classes R_F Values	Max., $m\mu$ (CCl_4) Material Sepd. into Classes	Paper Chromatography Propylene Glycol System: Spots from class sepn. R_F values	Paper Chromatography Propylene Glycol System: Authentic individual compounds, R_F values	Paper Chromatography Vaseline System: Spots from class sepn. R_F values	Paper Chromatography Vaseline System: Authentic individual compounds R_F values
Alkanal C_1	330	0.35	340–6	0.21	0.21		
Alk-2-enal C_3	354			0.44	0.44		
Alkanal C_2	343	0.35	346–9	0.42	0.38		
Alk-2-enal C_3	354				0.42		
Alk-2,4-dienal C_5	370–5	0.22	365–70	0.50	0.50		
2-Alkanone C_3	349	0.54	343–6	0.65	0.66		
Alkanal C_3	343				0.56		
Alk-2-enal C_4	358–61	0.35	358	0.59	0.56		
Alk-2,4-dienal C_6	380	0.21	370–5	0.60	0.61		
2-Alkanone C_3	349	0.48	346–9	0.73	0.66		
Alkanal C_4	343				0.73		
Alk-2-enal C_5	365	0.34	355–8	0.78	0.76		
Alk-2,4-dienal C_7	380	0.22	375–80	0.80	0.80		
Alkanal C_3	343	0.47	343	0.56	0.56		
Alk-2-enal C_4	358–61	0.34	358–65	0.56	0.56		
Alk-2,4-dienal C_6	380	0.22	375–80	0.61	0.61		
2-Alkanone C_4	349–52	0.57	349	0.83	0.85		
Alkanal C_4	343–6	0.47	343	0.73	0.72		
Alk-2-enal C_5	365	0.37	358–65	0.75	0.73		
Alk-2,4-dienal C_7	380	0.24	375–80	0.79	0.80		
2-Alkanone C_4	349–52	0.58	349	0.85	0.85		
Alkanal C_5	343–6	0.50	346–9	0.83	0.83		
Alk-2-enal C_6	365	0.38	361–5	0.86	0.86		
Alk-2,4,-dienal C_8	380	0.25	375–80	0.91	0.91		
2-Alkanone C_8	352	0.58	352			0.59	0.58
Alkanal C_9	343–6	0.48	346			0.58	0.58
Alk-2-enal C_{10}	365	0.36	361			0.56	0.56
Alk-2,4-dienal C_{12}	380	0.24	375–80			0.48	0.47
2-Alkanone C_9	352	0.55	349–52			0.48	0.48
Alkanal C_9	343–6	0.45	346			0.58	0.58
Alk-2-enal C_{10}	365	0.31	358–61			0.54	0.53
2-Alkanone C_9	352	0.55	349–52			0.49	0.48
Alkanal C_{10}	346	0.45	346			0.52	0.52
Alk-2-enal C_{11}	365	0.36	358–61			0.47	0.46

Table IV. Separation of Mixture of Four Homologous Series

Mixture of Members of 4 Homologous Series	Paper Chromatography: Untreated paper petm. Et_2O R_F values spots	Propylene Glycol–Skellysolve C–Methanol: R_F values unknown mixture	Propylene Glycol–Skellysolve C–Methanol: R_F values mixture authentic	Authentic Compounds Mixture	Unseparated Spots from Propylene Glycol System	Paper Chromatography, Vaseline–Aqueous Methanol: R_F values unknown mixture	Paper Chromatography, Vaseline–Aqueous Methanol: R_F values authentic mixture	Authentic Compounds Mixture
2-Alkanone C_4–C_9, C_{11}, C_{13} Alkanal C_3–C_{14} Alk-2-enal C_3–C_{12} Alk-2,4-dienal C_5–C_{12}	0.55	1.00				0.70	0.71	C_6-2-alkanone
		0.92	0.92	C_5 2-alkanone		0.63	0.64	C_7
		0.79	0.77	C_4		0.56	0.56	C_8
						0.49	0.50	C_9
						0.33	0.35	C_{11}
						0.21	0.23	C_{13}
	0.46	1.00				0.73	0.73	C_7-alkanal
		0.92	0.91	C_6-alkanal		0.65	0.66	C_8
		0.83	0.82	C_5		0.58	0.58	C_9
		0.73	0.71	C_4		0.51	0.51	C_{10}
		0.55	0.55	C_3		0.42	0.43	C_{11}
						0.36	0.36	C_{12}
						0.29	0.29	C_{13}
						0.22	0.23	C_{14}
	0.35	1.00					0.74	C_7-alk-2-enal
		0.91	0.91	C_7-alk-2-enal		0.67	0.68	C_8
		0.79	0.79	C_6		0.61	0.62	C_9
		0.67	0.65	C_5		0.55	0.55	C_{10}
		0.50	0.48	C_4		0.47	0.48	C_{11}
		0.39	0.38	C_3		0.39	0.41	C_{12}
	0.24	1.00					0.66	C_9-alk-2,4-dienal
		0.92	0.92	C_9-alk-2,4-dienal		0.59	0.60	C_{10}
		0.79	0.80	C_8	M	0.51	0.53	C_{11}
		0.67	0.68	C_7		0.44	0.45	C_{12}
		0.50	0.49	C_6				
		0.42	0.43	C_5				

Table V. Separation of Complete Mixture of Four Homologous Series

Mixture of Members of 4 Homologous Series	Paper Chromatography: Untreated paper petm. Et_2O R_F values of spots	Paper Chromatography: Vaseline–Aqueous Methanol R_F Values: Unknown mixture	Vaseline–Aqueous Methanol R_F Values: Authentic mixture	Authentic Compounds Mixture	Unseparated Spots from Vaseline System	Paper Chromatography, Propylene Glycol–Skelly C–Methanol R_F Values: Unknown mixture	Propylene Glycol–Skelly C–Methanol R_F Values: Authentic mixture	Authentic Compounds Mixture	Paper Chromatography, Propylene Glycol–Skelly C–Methanol R_F Values, Authentic Mixture	Authentic Compounds Mixture
		0.91				0.91	0.92	C_5-2-alkanone		
		0.71	0.71	C_6-2-alkanone		0.79	0.77	C_4		
		0.64	0.64	C_7			0.61	C_3		
	0.59	0.57	0.56	C_8						
		0.50	0.50	C_9						
		0.35	0.35	C_{11}						
		0.22	0.23	C_{13}						
						0.91	0.92	C_6-alkanal	0.91	C_6 alkanal
						0.83	0.81	C_5	0.82	C_5
		0.92				0.72		C_4	0.71	C_4
		0.73	0.73	C_7-alkanal		0.56	0.70	C_3-2 alkanone		
		0.66	0.67	C_8			0.55	C_3-alkanal	0.55	C_3
2-Alkanone		0.59	0.58	C_9			0.35	C_2	0.35	C_2
C_3–C_9, C_{11}, C_{13}	0.47	0.52	0.52	C_{10}			0.21	C_1	0.21	C_1
Alkanal		0.44	0.44	C_{11}						
C_1–C_{14}		0.36	0.36	C_{12}						
Alk-2-enal		0.30	0.30	C_{13}		0.81	0.80	C_6-alk-2-enal	0.80	C_6 alk-2-enal
C_3–C_{12}		0.23	0.23	C_{14}		0.69	0.67	C_5	0.67	C_5
Alk-2,4-dienal						0.52	0.52	C_4	0.52	C_4
C_5–C_{12}		0.91						C_3	0.35	C_3
		0.74	0.74	C_7-alk-2-enal		0.39	0.39	C_2-alkanal		
		0.67	0.68	C_8		0.21	0.21	C_1		
	0.35	0.61	0.62	C_9						
		0.54	0.55	C_{10}						
		0.48	0.48	C_{11}						
		0.41	0.41	C_{12}						
						0.80	0.80	C_8-alk-2,4-dienal		
		0.90				0.70	0.68	C_7		
		0.65	0.66	C_9-alk-2,4-dienal		0.52	0.49	C_6		
	0.23	0.60	0.60	C_{10}		0.44	0.44	C_5		
		0.53	0.53	C_{11}						
		0.45	0.45	C_{12}						

These methods appear adapted to quantitative application in spite of the small quantities involved, and the sensitivity of the compounds. 2,4-Dienals particularly appeared unstable, and fractions left exposed to light and air decreased in absorbance values and in absorption maxima (*17*). In paper chromatography there was always some material that remained in the origin, particularly on untreated paper. Nonmobile material was much smaller in amount on propylene glycol– and vaseline-impregnated paper. A mixture was made up of 2,4-dinitrophenylhydrazones of methyl ketones (C_4 to C_9, C_{11} and C_{13}), saturated aldehydes (C_3 to C_{14}), 2-enal (C_3 to C_{12}), and 2,4-dienal (C_5 to C_{12}) and separated into classes by chromatography on untreated paper. This mixture contained, on the basis of maximum absorbance, 24.6, 20.8, 24.1, and 30.5%, respectively. Proportions found were 25.3, 24.6, 24.0, and 26.1%, respectively. Total recovery of mobile material was 70.9%; this was equivalent to recoveries of 73.0, 81.0, 70.7, and 59.9%, respectively. Nonmobile material had a maximum at 346 to 52 mμ and amounted to 22.4% of the total amount of material recovered. In another experiment the four classes were each applied separately to untreated paper and chromatographed. Recoveries of mobile material were 86, 84, 66, and 74%, respectively. Material left at the origins made total recoveries 95, 93, 87, and 91%, respectively. The maxima of these nonmobile materials were 320 to 46, 320, 340 to 46, and 352 to 58 mμ. The quantitative application of these procedures requires further study.

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LITERATURE CITED

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